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Patentanmeldung Nr. Patent application No. Demande de brevet n°

94200721.2

PRIORITY DOCUMENT

Der Präsident des Europäischen Patentamts;
im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

D. HADFORD

Den Haag, den
The Hague, 24/03/95
La Haye, le

Eur päisches Patentamt
Eur pean Patent Office
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Blatt 2 d r B scheinigung
She t 2 f th c rtificate
Page 2 de l'attestation

Anmeldung Nr:
Application no:
Demande n°: 94200721.2

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Date of filing:
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Anmelder:
Applicant(s):
Demandeur(s):
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NL-3584 CS Utrecht
NETHERLANDS

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Pharmaceutical composition for the treatment and prevention of inflammatory diseases and active components of such compositions

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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Bemerkungen:
Remarks:
Remarques:

SEQ ID No. 1

Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu
5 10 15

Glu Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu
20 25 30

Gly Pro Gly Lys Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala
35 40 45

Pro Thr Ile Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu
50 55 60

Leu Glu Asp Pro Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu
65 70 75

Val Ala Lys Lys Thr Asp Asp Val Ala Gly Asp Gly Thr Thr Thr
80 85 90

Ala Thr Val Leu Ala Gln Ala Leu Val Arg Glu Gly Leu Arg Asn
95 100 105

Val Ala Ala Gly Ala Asn Pro Leu Gly Leu Lys Arg Gly Ile Glu
110 115 120

Lys Ala Val Glu Lys Val Thr Glu Thr Leu Leu Lys Gly Ala Lys
125 130 135

Glu Val Glu Thr Lys Glu Gln Ile Ala Ala Thr Ala Ala Ile Ser
140 145 150

Ala Gly Asp Gln Ser Ile Gly Asp Leu Ile Ala Glu Ala Met Asp
155 160 165

Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu Glu Ser Asn Thr
170 175 180

Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg Phe Asp Lys
185 190 195

Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Pro Glu Arg Gln Glu
200 205 210

Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val
215 220 225

Ser Thr Val Lys Asp Leu Leu Pro Leu Leu Glu Lys Val Ile Gly
230 235 240

Ala Gly Lys Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly Glu
245 250 255

Ala Leu Ser Thr Leu Val Val Asn Lys Ile Arg Gly Thr Phe Lys
260 265 270

Ser Val Ala Val Lys Ala Pro Gly Phe Gly Asp Arg Arg Lys Ala
275 280 285

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Met Leu Gln Asp Met Ala Ile Leu Thr Gly Gly Gln Val Ile Ser
                290                 295                 300

Glu Glu Val Gly Leu Thr Leu Glu Asn Ala Asp Leu Ser Leu Leu
            305                 310                 315

Gly Lys Ala Arg Lys Val Val Val Thr Lys Asp Glu Thr Thr Ile
            320                 325                 330

Val Glu Gly Ala Gly Asp Thr Asp Ala Ile Ala Gly Arg Val Ala
            335                 340                 345

Gln Ile Arg Gln Glu Ile Glu Asn Ser Asp Ser Asp Tyr Asp Arg
            350                 355                 360

Glu Lys Leu Gln Glu Arg Leu Ala Lys Leu Ala Gly Gly Val Ala
            365                 370                 375

Val Ile Lys Ala Gly Ala Ala Thr Glu Val Glu Leu Lys Glu Arg
            380                 385                 390

Lys His Arg Ile Glu Asp Ala Val Arg Asn Ala Lys Ala Ala Val
            395                 400                 405

Glu Glu Gly Ile Val Ala Gly Gly Gly Val Thr Leu Leu Gln Ala
            410                 415                 420

Ala Pro Thr Leu Asp Glu Leu Lys Leu Glu Gly Asp Glu Ala Thr
            425                 430                 435

Gly Ala Asn Ile Val Lys Val Ala Leu Glu Ala Pro Leu Lys Gln
            440                 445                 450

Ile Ala Phe Asn Ser Gly Leu Glu Pro Gly Val Val Ala Glu Lys
            455                 460                 465

Val Arg Asn Leu Pro Ala Gly His Gly Leu Asn Ala Gln Thr Gly
            470                 475                 480

Val Tyr Glu Asp Leu Leu Ala Ala Gly Val Ala Asp Pro Val Lys
            485                 490                 495

Val Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Gly Leu
            500                 505                 510

Phe Leu Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu Lys Glu
            515                 520                 525

Lys Ala Ser Val Pro Gly Gly Gly Asp Met Gly Gly Met Asp Phe
            530                 535                 540
```

SEQ ID NO. 2

SEQ 2/1

The alignment was done on 4 Protein sequences.
Character to show that a position in the alignment is perfectly conserved: '*'
Character to show that a position is well conserved: '.'

Alignment

```
P60$HUMAN   MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVKFGADARALMLQGVDLLADA     50
P60$RAT     ------------------A--------KDVKFGADARALMLQGVDLLADA     24
P60$MOUSE   ------------------APHLTRAYAKDVKFGADARALMLQGVDLLADA     32
MBAA        M-------------------------AKTIAYDEEARRGLERGLNALADA     25
                                      *.. ....**  . .*.. ****

P60$HUMAN   VAVTMGPKGRTVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQD    100
P60$RAT     VAVTMGPKGRTVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQD     74
P60$MOUSE   VAVTMGPKGRTVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQD     82
MBAA        VKVTLGPKGRNVVLEKKWGAPTITNDGVSIAKEIELEDPYEKIGAELVKE     75
            * **.*****.*..*..**.*..*.***..**.*.*.* *..***.**..

P60$HUMAN   VANNTNEEAGDGTTTATVLARSIAKEGFEKISKGANPVEIRRGVMLAVDA    150
P60$RAT     VANNTNEEAGDGTTTATVLARSIAKEGFEKISKGANPVEIRRGVMLAVDA    124
P60$MOUSE   VANNTNEEAGDGTTTATVLARSIAKEGFEKISKGANPVEIRRGVMLAVDA    132
MBAA        VAKKTDDVAGDGTTTATVLAQALVREGLRNVAAGANPLGLKRGIEKAVEK    125
            **..*.. ************.....**. ... ****....**.  **.

P60$HUMAN   VIAELKKQSKPVTTPEEIAQVATISANGDKEIGNIISDAMKKVGRKGVIT    200
P60$RAT     VIAELKKQSKPVTTPEEIAQVATISANGDKDIGNIISDAMKKVGRKGVIT    174
P60$MOUSE   VIAELKKQSKPVTTPEEIAQVATISANGDKDIGNIISDAMKKVGRKGVIT    182
MBAA        VTETLLKGAKEVETKEQIAATAAISA-GDQSIGDLIAEAMDKVGNEGVIT    174
            *...* * .* *.* *.**..*.*** **..**..*..**.***..****

P60$HUMAN   VKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQKCEFQDAYVLLSEKK    250
P60$RAT     VKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQKCEFQDAYVLLSEKK    224
P60$MOUSE   VKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQKCEFQDAYVLLSEKK    232
MBAA        VEESNTFGLQLELTEGMRFDKGYISGYFVTDPERQEAVLEDPYILLVSSK    224
            *....*.. .**..***.**.**** **..... *.  ..*.*.** ..*
```

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P60$HUMAN   ISSIQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVLNRLKVGLQVVAV  300
P60$RAT     ISSVQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVLNRLKVGLQVVAV  274
P60$MOUSE   FSSVQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVLNRLKVGLQVVAV  282
MBAA        VSTVKDLLLPLLEKVIGAGKPLLIIAEDVEGEALSTLVVNKIRGTFKSVAV  274
             *......* ** . . ***.*******.********.*... ... ***

P60$HUMAN   KAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIV  350
P60$RAT     KAPGFGDNRKNQLKDMAIATGGAVFGEEGLNLNLEDVQAHDLGKVGEVIV  324
P60$MOUSE   KAPGFGDNRKNQLKDMAIATGGAVFGEEGLNLNLEDVQAHDLGKVGEVIV  332
MBAA        KAPGFGDRRKAMLQDMAILTGGQVISEE-VGLTLENADLSLLGKARKVVV  323
            *******.**. *.**** ***.*..** ..*.**...   ***. .*.*

P60$HUMAN   TKDDAMLLKGKGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDG  400
P60$RAT     TKDDAMLLKGKGDKAHIEKRIQEITEQLDITTSEYEKEKLNERLAKLSDG  374
P60$MOUSE   TKDDAMLLKGKGDKAHIEKRIQEITEQLDITTSEYEKEKLNERLAKLSDG  382
MBAA        TKDETTIVEGAGDTDAIAGRVAQIRQEIENSDSDYDREKLQERLAKLAGG  373
            ***.. ...* **.. *. *...* .... ..*.*..***.******..*

P60$HUMAN   VAVLKVGGTSDVEVNEKKDRVTDALNATRAAVEEGIVLGGGCALLRCIPA  450
P60$RAT     VAVLKVGGTSDVEVNEKKDRVTDALNATRAAVEEGIVLGGGCALLRCIPA  424
(  0$MOUSE  VAVLKVGGTSDVEVNEKKDRVTDALNATRAAVEEGIVLGGGCALLRCIPA  432
MBAA        VAVIKAGAATEVELKERKHRIEDAVRNAKAAVEEGIVAGGGVTLLQAAPT  423
            ***.*.*....**..*.*.*..**.....******** *** .**.  *.

P60$HUMAN   LDSLTPANEDQKIGIEIIKRTLKIPAMTIAKNAGVEGSLIVEKIMQSSSE  500
P60$RAT     LDSLKPANEDQKIGIEIIKRALKIPAMTIAKNAGVEGSLIVEKILQSSSE  474
P60$MOUSE   LDSLKPANEDQKIGIEIIKRALKIPAMTIAKNAGVEGSLIVEKILQSSSE  482
MBAA        LDELK-LEGDEATGANIVKVALEAPLKQIAFNSGLEPGVVAEKVRNLPAG  472
            **.*.  ..*. .* .*.* .*. * . ** *.*.* ....**. . ...

P60$HUMAN   VGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEVVVTEIP  550
P60$RAT     VGYDAMLGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEAVVTEIP  524
P60$MOUSE   VGYDAMLGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEAVVTEIP  532
MBAA        HGLNAQTGVYEDLLAAGVADPVKVTRSALQNAASIAGLFLTTEAVVADKP  522
             * .*  * . .... *. **.**.*.** .**..*.*. *.*.**.. *

P60$HUMAN   KEEKDPGMGAMGGMGGGMGGGMF   573
P60$RAT     KEEKDPGMGAMGGMGGGMGGGMF   547
P60$MOUSE   KEEKDPGMGAMGGMGGGMGGGMF   555
(  3AA      EKEKASVPG-----GGDMGGMDF   540
            ..**..  *     **.***  *
```

Consensus length: 573
Identity : 254 (44.3%)
Similarity: 211 (36.8%)

SEQ ID. [redacted].3

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1==========================================================
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****
* TRANSLATION OF NUCLEIC ACID SEQUENCE OF THE MYCOB. BOVIS BCG HSP65 GENE*
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                    ----------
      580         590         600         610         620
       |           |           |           |           |
ATG GCC AAG ACA ATT GCG TAC GAC GAA GAG GCC CGT CGC GGC CTC GAG CGG GGC
 M   A   K   T   I   A   Y   D   E   E   A   R   R   G   L   E   R   G

630         640         650         660         670         680
 |           |           |           |           |           |
TTG AAC GCC CTC GCC GAT GCG GTA AAG GTG ACA TTG GGC CCC AAG GGC CGC AAC
 L   N   A   L   A   D   A   V   K   V   T   L   G   P   K   G   R   N

      690         700         710         720         730
       |           |           |           |           |
GTC GTC CTG GAA AAG AAG TGG GGT GCC CCC ACG ATC ACC AAC GAT GGT GTG TCC
 V   V   L   E   K   K   W   G   A   P   T   I   T   N   D   G   V   S

 740         750         760         770         780         790
  |           |           |           |           |           |
ATC GCC AAG GAG ATC GAG CTG GAG GAG CTG GAG GAT CCG TAC GAG GCC GAG CTG
 I   A   K   E   I   E   L   E   E   L   E   D   P   Y   E   A   E   L

      800         810         820         830         840
       |           |           |           |           |
GTC AAA GAG GTA GCC AAG AAG ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG ACG
 V   K   E   V   A   K   K   T   D   D   V   A   G   D   G   T   T   T
```

72

84

90

```
    850         860         870         880         890
     |           |           |           |           |
GCC ACC GTG CTG GCC CAG GCG TTG GTT CGC CAG GGC CTG CGC AAC GTC GCG GCC
A   T   V   L   A   Q   A   L   V   R   Q   G   L   R   N   V   A   A
                95                                                    102

900         910         920         930         940         950
 |           |           |           |           |           |
GGC GCC AAC CCG CTC GGT CTC AAA CGC GGC ATC GAA AAG GCC GTG GAG AAG GTC
G   A   N   P   L   G   L   K   R   G   I   E   K   A   V   E   K   V
                                                                    112

        960         970         980         990         1000
         |           |           |           |           |
ACC GAG ACC CTG CTC AAG GGC GCC AAG GAG GTC GAG ACC AAG GAG CAG ATT GCG
T   E   T   L   L   K   G   A   K   E   V   E   T   K   E   Q   I   A
                                                                    124

1010        1020        1030        1040        1050        1060
 |           |           |           |           |           |
GCC ACC GCA GCG ATT TCG GCG GGT GAC CAG TCC ATC GGT GAC CTG ATC GCC GAG
A   T   A   A   I   S   A   G   D   Q   S   I   G   D   L   I   A   E   136

        1070        1080        1090        1100        1110
         |           |           |           |           |
GCG ATG GAC AAG GTG GGC AAC GAG GGC GTC ATC ACC GTC GAG GAG TCC AAC ACC
A   M   D   K   V   G   N   E   G   V   I   T   V   E   E   S   N   T   148

1120        1130        1140        1150        1160
 |           |           |           |           |
TTT GGG CTG CAG CTC GAG CTC ACC GAG GGT ATG CGG TTC GAC AAG GGC TAC ATC
F   G   L   Q   L   E   L   T   E   G   M   R   F   D   K   G   Y   I   159
```

1170 1180 1190 1200 1210 1220

TCG GGG TAC TTC GTG ACC GAC CCG GAG CGT CAG GAG GCG GTC CTG GAG GAC CCC

S G Y F V T D P E R Q E A V L E D P 216

1230 1240 1250 1260 1270

TAC ATC CTG CTG GTC AGC TCC AAG GTG TCC ACT GTC AAG GAT CTG CTG CCG CTG

Y I L L V S S K V S T V K D L L P L 23(

1280 1290 1300 1310 1320 1330

CTC GAG AAG GTC ATC GGA GCC GGT AAG CCG CTG CTG ATC ATC GCC GAG GAC GTC

L E K V I G A G K P L L I I A E D V 25(

1340 1350 1360 1370 1380

GAG GGC GAG GCG CTG TCC ACC CTG GTC GTC AAC AAG ATC CGC GGC ACC TTC AAG

E G E A L S T L V V N K I R G T F K 27

1390 1400 1410 1420 1430

TCG GTG GCG GTC AAG GCT CCC GGC TTC GGC GAC CGC CGC AAG GCG ATG CTG CAG

S V A V K A P G F G D R R K A M L Q 2(

1440 1450 1460 1470 1480 1490

GAT ATG GCC ATT CTC ACC GGT GGT CAG GTG ATC AGC GAA GAG GTC GGC CTG ACG

D M A I L T G G Q V I S E E V G L T 3(

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      1500          1510          1520          1530          1540
       |             |             |             |             |
CTG GAG AAC GCC GAC CTG TCG CTG CTA GGC AAG GCC CGC AAG GTC GTG GTC ACC
 L   E   N   A   D   L   S   L   L   G   K   A   R   K   V   V   V   T  324

      1550          1560          1570          1580          1590          1600
       |             |             |             |             |             |
AAG GAC GAG ACC ACC ATC GTC GAG GGC GCC GGT GAC ACC GAC GCC ATC GCC GGA
 K   D   E   T   T   I   V   E   G   A   G   D   T   D   A   I   A   G  342

      1610          1620          1630          1640          1650
       |             |             |             |             |
CGA GTG GCC CAG ATC CGC CAG GAG ATC GAG AAC AGC GAC TCC GAC TAC GAC CGT
 R   V   A   Q   I   R   Q   E   I   E   N   S   D   S   D   Y   D   R  360

      1660          1670          1680          1690          1700
       |             |             |             |             |
GAG AAG CTG CAG GAG CGG CTG GCC AAG CTG GCC GGT GGT GTC GCG GTG ATC AAG
 E   K   L   Q   E   R   L   A   K   L   A   G   G   V   A   V   I   K  378

1710          1720          1730          1740          1750          1760
 |             |             |             |             |             |
GCC GGT GCC GCC ACC GAG GTC GAA CTC AAG GAG CGC AAG CAC CGC ATC GAG GAT
 A   G   A   A   T   E   V   E   L   K   E   R   K   H   R   I   E   D  396

      1770          1780          1790          1800          1810
       |             |             |             |             |
GCG GTT CGC AAT GCC AAG GCC GCC GTC GAG GAG GGC ATC GTC GCC GGT GGG GGT
 A   V   R   N   A   K   A   A   V   E   E   G   I   V   A   G   G   G  414
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1820          1830          1840          1850          1860          1870
 |             |             |             |             |             |
GTG ACG CTG TTG CAA GCG GCC CCG ACC CTG GAC GAG CTG AAG CTC GAA GGC GAC
 V   T   L   L   Q   A   A   P   T   L   D   E   L   K   L   E   G   D

        1880          1890          1900          1910          1920
         |             |             |             |             |
GAG GCG ACC GGC GCC AAC ATC GTG AAG GTG GCG CTG GAG GCC CCG CTG AAG CAG
 E   A   T   G   A   N   I   V   K   V   A   L   E   A   P   L   K   Q

      1930          1940          1950          1960          1970
       |             |             |             |             |
ATC GCC TTC AAC TCC GGG CTG GAG CCG GGC GTG GTG GCC GAG AAG GTG CGC AAC
 I   A   F   N   S   G   L   E   P   G   V   V   A   E   K   V   R   N

1980          1990          2000          2010          2020          2030
 |             |             |             |             |             |
CTG CCG GCT GGC CAC GGA CTG AAC GCT CAG ACC GGT GTC TAC GAG GAT CTG CTC
 L   P   A   G   H   G   L   N   A   Q   T   G   V   Y   E   D   L   L

        2040          2050          2060          2070          2080
         |             |             |             |             |
GCT GCC GGC GTT GCT GAC CCG GTC AAG GTG ACC CGT TCG GCG CTG CAG AAT GCG
 A   A   G   V   A   D   P   V   K   V   T   R   S   A   L   Q   N   A

2090          2100          2110          2120          2130          2140
 |             |             |             |             |             |
GCG TCC ATC GCG GGG CTG TTC CTG ACC ACC GAG GCC GTC GTT GCC GAC AAG CCG
 A   S   I   A   G   L   F   L   T   T   E   A   V   V   A   D   K   P
```

450

```
          2150        2160        2170        2180        2190
           |           |           |           |           |
GAA AAG GAG AAG GCT TCC GTT CCC GGT GGC GGC GAC ATG GGT GGC ATG GAT TTC
E   K   E   K   A   S   V   P   G   G   G   D   M   G   G   M   D   F
```

```
    2200
     |
TGA CCC
-   P
```

Pharmaceutical composition for the treatment and prevention of inflammatory diseases and active components of such compositions

The invention pertains to polypeptides containing a part of the amino acid sequence of the heat shock protein hsp65 of *Mycobacterium tuberculosis* which polypeptides are capable of immunizing against arthritis and other inflammatory diseases and/or curing such diseases, as well as to nucleotide sequences encoding such polypeptides, cells and microorganisms expressing such polypeptides and pharmaceutical and diagnostic compositions containing such polypeptides.

It has been found that experimental arthritis can be induced by administering killed *Mycobacterium tuberculosis*. It was also found that immunisation with mycobacterial hsp65 (a member of the hsp60 family of heat shock proteins) induces resistance to arthritis. Also mycobacterial hsp65 itself was capable of suppressing developing arthritis.

T cell epitopes of mycobacterial hsp65 that are recognised after hsp65 immunisation were analysed. Immunisation with hsp65 led to the recognition of a series of nine distinct dominant and subdominant epitopes. These are the aminoacid sequences 91-100, 180-188, 216-225, 226-235, 256-265, 386-400, 396-405, 446-455 and 511-520 of the mycobacterial hsp as shown in SEQ ID No. 1.

It was found that immunisation of rats with a peptide corresponding to sequence 256-265 of SEQ ID No.1 induced strong protection against induction, seven days later, of adjuvant arthritis (AA). This finding was confirmed when using peptide 256-270. Immunisation with a peptide corresponding to sequence 91-100 of SEQ ID No.1 induced moderate protection, whereas immunisation with peptides corresponding to the other epitopes produces little or no protection against adjuvant arthritis.

The T cell line H.52, originally generated from hsp65 immunised rats and specific for epitope 256-265 also showed a protective effect on AA development when injected i.v. at the time of administration of *Mycobacterium tuberculosis*.

It is concluded that protective epitopes in hsp65 are located at positions where at least 5 aminoacids are in the same relative position as the same aminoacids in a T cell epitope of hsp65 that contains at least 4 consecutive aminoacids which are identical with the corresponding mammalian hsp60 aminoacids. Mammalian hsp includes human, rat and mouse hsp. The human, rat, mouse and mycobacterial hsp60/hsp65 aminoacid

sequences are depicted in one letter code in SEQ ID No. 2. The aminoacids which are identical are also shown in SEQ ID No. 2.

The polypeptides are especially those having 5 aminoacids which are in the same relative position as the same aminoacids in one of the sequences 81-100 and 241-270 of SEQ ID No. 1, more particularly having at least 5 aminoacids which are in the same relative position as the same aminoacids in one of the sequences 84-95 and 256-265 of SEQ ID No. 1. Withe preference, the polypeptides comprise at least 7 aminoacids with the same relative positions as those in the hsp65 T cell epitopes. Those epitopes are especially those which have at least 4 consecutive aminoacids which are identical with the corresponding mammalian hsp60 aminoacids. Examples of suitable polypeptide comprise the sequences [Ala Thr Val Leu Ala], [Ala Leu Ser Thr Leu] and [Leu Ser Thr Leu Val]. In particular, the polypeptide comprises 5-30 aminoacids of the amino acid sequence of hsp65; these hsp65 aminoacids may be coupled to other sequences, such as spacer sequences or fused peptide sequences.

The polypeptides are suitable for protecting against inflammatory diseases, including autoimmune diseases, diabetes, arthritide diseases, atherosclerosis, multiple sclerosis, and myasthenia gravis.

The invention also concerns polypeptide analogues which exhibit the immunological properties of the polypeptides described above, but which contain one or chemical modifications. Such polypeptide analogues, also referred to as peptidomimetics, can e.g. consist of units corresponding to the aminoacid residues of the polypeptides described above, wherein essentially the same side groups are present, but wherein the backbone contains modifications such as substitution of an amide group (CO-NH) by another group such as CH=CH, CO-O, CO-CH_2 or CH_2-CH_2. Other modifications, such as substitutions of an aminoacid by a similar natural, or non-natural aminoacid are also envisaged.

The invention furthermore relates to pharmaceutical compositions suitable for protection against or treatment of an inflammatory disease, including autoimmune diseases, diabetes, arthritide diseases, multiple sclerosis and myasthenia gravis, containing a polypeptide as described above or a nucleotide sequence, an expression system, a cell (eukaryotic) or microorganism corresponding to and/or encoding such polypeptide. The composition may be in the form of a vaccine; it can then also contain a conventional adjuvant, such as Freund's complete or incomplete adjuvant or other adjuvant, and/or carrier materials and other additives.

The composition may also be in the form of a medicine suitable for curing developing or developed inflammatory diseases; it contains conventional additives and excipients. As a treatment composition, it may also contain an antibody against the polypeptides described above.

The invention also relates to diagnostics means and methods based on the polypeptides described above, or the corresponding antibodies or nucleotide sequences (probes).

Figure 1 shows modulation of AA using epitope-specific T cell lines (5,000,000 T cells i.v. in PBS or PBS alone at the time of AA induction using 0.5 mg *Mycobacterium tuberculosis* in 100 µl IFA i.d. at the base of the tail). Results with lines H.46 (226-235) and H.52 (256-265) are shown. Lines corresponding to sequences 180-188 and 216-225 did not show a significant effect.

Figure 2 shows modulation of CP20961-induced arthritis in the same way. CP20961 is a lipoidal amine.

SEQ. ID No 3. contains the nucleotide sequence and aminoacid sequence (1-letter) of hsp65. Sequences 84-95 and 256-270 are sequences corresponding to protective polypeptides.

Claims

1. Polypeptide containing a part of the amino acid sequence of the heat shock protein hsp65 of *Mycobacterium tuberculosis* as depicted in SEQ ID No. 1, comprising at least 5 aminoacids which are in the same relative position as the same aminoacids in a T cell epitope of hsp65 that contains at least 4 consecutive aminoacids which are identical with the corresponding mammalian hsp60 aminoacids.

2. Polypeptide according to claim 1, wherein the polypeptide comprises at least 5 aminoacids which are in the same relative position as the same aminoacids in one of the sequences 81-100 and 241-270 of SEQ ID No. 1.

3. Polypeptide according to claim 2, wherein the polypeptide comprises at least 5 aminoacids which are in the same relative position as the same aminoacids in one of the sequences 84-95 and 256-265 of SEQ ID No. 1.

4. Polypeptide according to any one of claims 1-3, wherein the polypeptide comprises 5-30 aminoacids of the amino acid sequence of hsp65.

5. Polypeptide analogue which exhibits the immunological properties of a peptide according to any one of claims 1-4, but which contains one or chemical modifications.

6. Nucleotide sequence encoding a polypeptide according to any one of claims 1-4.

7. Expression system capable of expressing a polypeptide according to any one of claims 1-4.

8. Microorganism containing an expression system according to claim 7.

9. Eukaryotic cell containing an expression system according to claim 7.

10. Cell expressing a receptor from a T cell activated by immunostimulation using a polypeptide according to any one of claims 1-5.

11. Antibody raised against a polypeptide according to any one of claims 1-5.

12. Pharmaceutical composition suitable for protection against or treatment of an inflammatory disease, including autoimmune diseases, diabetes, arthritide diseases, atherosclerosis, multiple sclerosis, myasthenia gravis, containing a polypeptide according to any one of claims 1-5, a nucleotide sequence according to claim 6, an expression system according to claim 7, a cell according to any one of claims 8-10, or an antibody according to claim 11.

13. Diagnostic composition containing a polypeptide according to any one of claims 1-5 or an antibody according to claim 11.

Figure 1

Modulation of AA by T cell lines

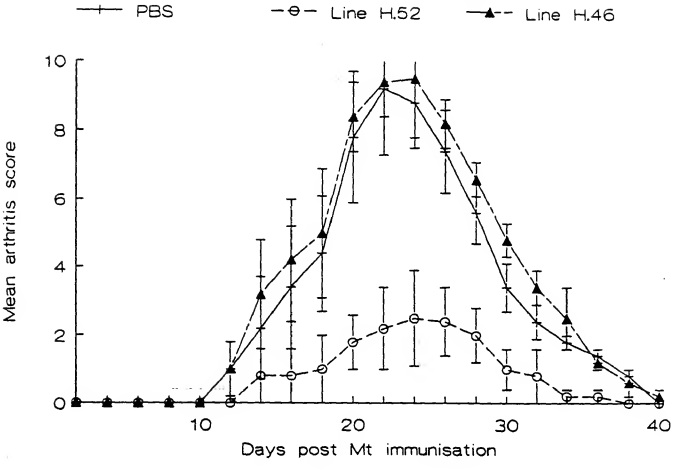

Figure 2
Modulation of CP20961-induced arthritis